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THE LARVAL CULTURE OF *Penaeus stylirostris* USING MODIFICATIONS OF THE GALVESTON LABORATORY TECHNIQUE[1]

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ABSTRACT

Preliminary observations on the larval culture of *Penaeus stylirostris* using modifications of the "Galveston Laboratory" technique included: 1) installing airlift pumps in spawning tanks; 2) treating water with ethylenedinitrilo tetra-acetic acid, disodium salt (EDTA); 3) using small UV germicidal lamps to eliminate contaminationg dinoflagellates from hatchery tanks; and 4) testing the use of two forms of bakers' yeast (*Saccharomyces cerevisiae*), compressed cake and active dry yeast (ADY), as replacement for frozen algae as larval foodstuff.

The design of the spawning tank helped to increase the hatching of fertile eggs from 0% to 80%. It was shown by bioassay testing that the UV light eliminated the dinoflagellate and that direct treatment of hatchery water with the germicidal lamp had no apparent detrimental effect on foodstuff or shrimp larvae. Bakers' ADY appeared to be more readily accepted than compressed yeast cake by shrimp larvae as foodstuff and was easier to prepare and monitor. Although yeast was an efficient replacement for frozen algae as foodstuff for larval shrimp, it took slightly longer for the total populations to metamorphose to postlarvae when yeast was fed.

INTRODUCTION

Initially, basic physical descriptions for the design of a penaeid shrimp hatchery were presented by Cook and Murphy (1969) and were updated by Mock and Murphy (1971). Later, Salser and Mock (1974) documented modifications to the system, including design of the rearing tanks, aeration and filtration equipment and the addition of equipment for concentrating and storing algae. More recently, Mock et al. (1974)

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elaborated upon the construction and use in hatchery situations of simple airlift pumps from schedule 40 plastic pipe. In the decade following the publication of Cook and Murphy (1969) and the documented modifications that followed, the penaeid shrimp hatchery system developed at the National Marine Fisheries Service, Southeast Fisheries Center, Galveston Laboratory, Galveston, Texas, has been copied and used extensively worldwide.

The hatchery technique used at the Galveston Laboratory has remained virtually unchanged for the past six years. The success by the Galveston Shrimp Maturation group in maturing and spawning the blue shrimp, *Penaeus stylirostris* (Brown et al. 1980), provided large quantities of viable eggs for more elaborate hatchery research. The intensified experimentation, however, defined a number of new problem areas in the penaeid shrimp maturation and hatchery systems. Chief among these problems were: 1) cost and time delay in producing large quantities of algae; 2) maintaining single cell suspension of algae in hatchery tanks; 3) dinoflagellate contamination in hatchery tanks; and 4) keeping developed fertilized eggs aerated and suspended in the water column before hatching.

The objectives of the study reported here were threefold. The first objective was to test bakers' yeast (either in dry form or compressed cake) as a supplement or replacement foodstuff for algae. Bakers's yeast (*Saccharomyces cerevisiae*) has been the subject of extensive morphological, cytological, physiological, and genetic studies (Cook 1958). Its use as a food for larval shrimp was first reported by Hudinaga and Kittaka (1966). They tested bakers' yeast with larval *P. japonicus* and found that "larvae fed on live yeast took three days to pass through zoea=1" and that as a foodstuff it was inferior to a variety of other foods tested. Kittaka (1969) applied for a patent of a system for the mass production of prawn larvae that included the feeding of "fresh bread yeast." According to his data, the bread yeast sustained good survival and growth through "zoea 2nd stage." Again, however, yeast was found to be inferior to a number of other foods tested. Marine yeast has been tested as a food for larval *P. japonicus* by Furukawa (1972) and more recently, Hirata et al. (1975) surmised, "A mixture of soy cake and diatoms or other suitable planktonic organisms, such as bread yeast, might provide an efficient diet for the mass culture of penaeid prawn larvae." The second objective was to test a flow-through UV-germicidal lamp as a treatment for eliminating dinoflagellates. The manufacturer's specifications indicated the lamp would eliminate ciliated protozoa, but it was unknown if the lamp would eliminate the unwanted dinoflagellate and if there would be any effect to the shrimp larvae. The third objective was to test an airlift pump system in spawning tanks to keep eggs suspended during hatching, thereby increasing hatching survival.

MATERIALS AND METHODS

SPAWNING TANKS

The experimental design used at Galveston for inducing mating and ovarian development has been described by Brown et al. (1980). Those females determined to be gravid and near spawning were removed from the large mating tank and placed singly in spawning tanks.

The fiberglass spawning tanks were 97.5 cm in diameter and 90 cm deep with a total volume of 400 liters and spawning volume of 140 liters.

A single, 3.8 cm airlift pump was suspended in the center of each tank (Fig. 1). The pumps were constructed from schedule 40 plastic pipe and were identical to those described by Mock (1974), with the exception that the 90° elbow on the top was omitted.

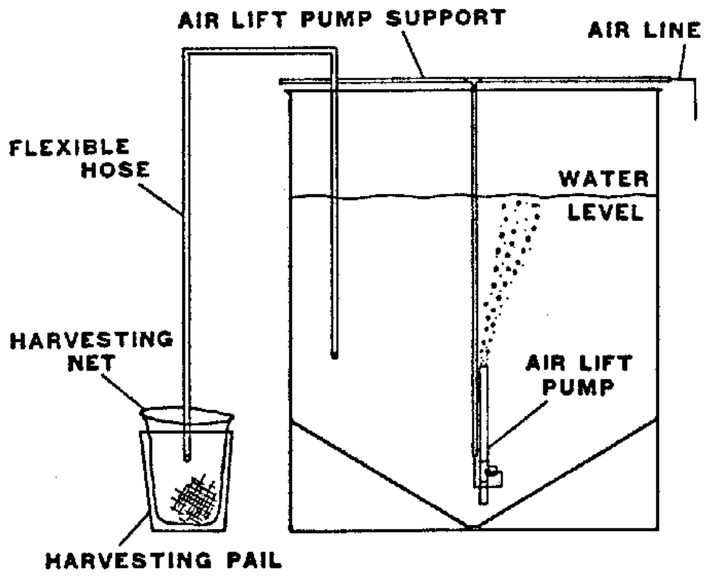

Figure 1. Spawning container and harvesting assembly.

Viable eggs and larval shrimp were removed from the spawning tank by siphoning through a flexible 2.5 cm diameter plastic hose and were collected in a 69 μ net. The net was placed in a plastic bucket and held so that the top of the net extended well above the top edge of the bucket (Fig. 1). The eggs and larvae in the net were transferred to the hatchery tank by inverting the net and flushing with seawater.

HATCHERY TANK

The 2000 liter conical fiberglass hatchery tanks have been described by Salser and Mock (1974) and were used for comparing frozen algae and active dry yeast as foodstuff for larval shrimp. In Experiment XI, a tank-mounted ultraviolet (UV) light (Hawaiian Marine Imports, Inc., Model AN-8*), at a germicidal intensity of 74,300 microwatts per cm^2 (2537 Angstroms) was installed to test its use for eliminating unwanted dinoflagellates from hatchery water.

BIOASSAY TANK

The bioassay tank (Fig. 2) was a fiberglass rectangle measuring 50 cm wide, 50 cm deep, and 82.5 cm long. Tests were performed with 50 liters of water. The control (Tank I) had no UV light, while Tank II had an AN-8 UV light. A small submersible plastic pump (2 liters/min)

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was placed inside Tank II and connected to the light. The water was aerated by a single airstone in each tank.

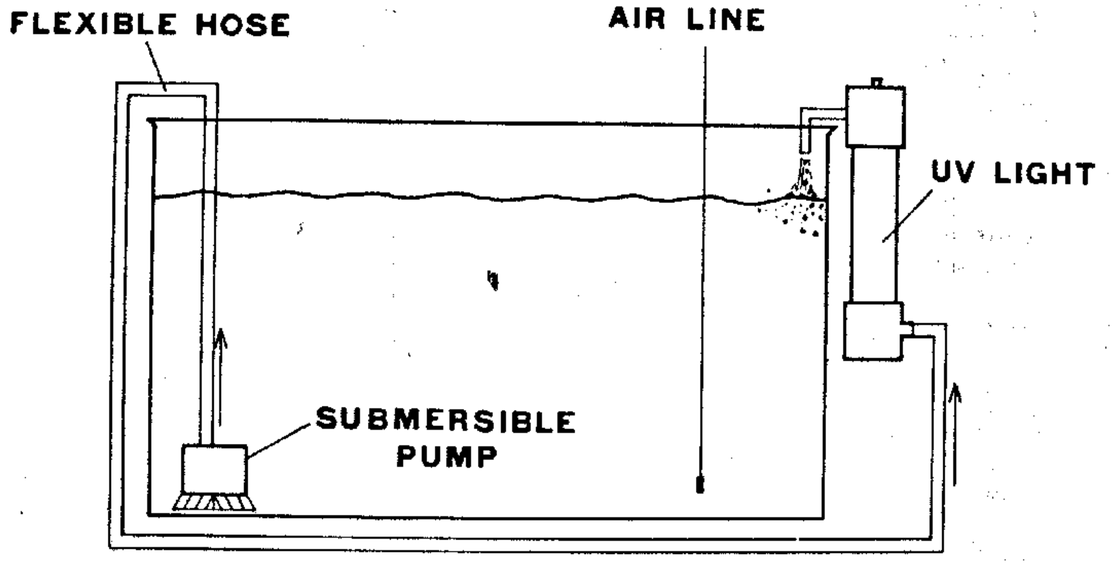

Figure 2. Bioassay tank assembly.

ALGAE PREPARATION

Algae used in this study were cultured at the Galveston Laboratory, concentrated, and frozen according to procedures described by Griffith et al. (1973). Frozen algae were thawed in deionized water before feeding and poured through a 69 μ net to eliminate clumps and any debris that might occur. The filtered algal solution was then poured into the tanks.

YEAST PREPARATION

Two forms of Fleischmann's bakers' yeast--compressed cake and active dry yeast--purchased at a local supermarket, were tested in this study. Both forms of yeast were prepared the same before feeding. One liter of deionized water (24-28°C) was set in motion with a magnetic stirrer and the yeast added slowly. The yeast cake was slowly crumbled by hand to enhance dissolving. The yeast mixture could be fed after mixing for 5 min or could be stored in the refrigerator to be fed at a later time. Yeast solutions stored in the refrigerator were stirred for 5 min with a magnetic stirrer before feeding.

A 16.8 g cake of compressed yeast dissolved in one liter of water yields a cell density of 100,000 cells per milliliter when placed in the 2000 liter hatchery tank. The same cell density is achieved with 7.0 g of ADY.

Artemia PREPARATION

Aquafauna *Artemia* cysts were used throughout this study and yielded consistently good hatching success (6 g sample of cysts yields 1.2 million nauplii). Six hundred grams of cysts in 800 liters of seawater (28

ppt, 28°C) were aerated vigorously for 24 hours. The entire contents of the tank were then strained through a 69 μ net and a nauplii density count made. The *Artemia* were frozen wide mouth plastic containers at a density of 4 million nauplii per container. This density yields 2 frozen *Artemia* per milliliter in the 2000 liter hatchery tank. The frozen blocks of *Artemia* can be removed intact from the containers before feeding. The unhatched cysts are all on the surface of the frozen block and can be removed by flushing with tap water. After the top layer of unhatched cysts has been removed, the frozen block is placed in the hatchery tank where it will thaw in about 5 min.

During this study *Artemia* cysts were also decapsulated, using the Clorox immersion technique, and stored in brine as described by Bruggeman and Sorgeloos (1979). This procedure was demonstrated to us at our laboratory by Etienne Bossuyt (University of Ghent, Ghent, Belgium) during August 1979.

SEAWATER PREPARATION

All seawater used in this study was filtered through a one μ cartridge filter and adjusted to the desired salinity with Instant Ocean. The water temperature was maintained with a thermostatically controlled electric immersion heater (100 v, 100 w). The filtered seawater was treated with EDTA (0.01 g/liter) and Erythromycin (5.0 mg/liter) for each experiment before introduction of the shrimp larvae.

RESULTS

SPAWNING

The Shrimp Maturation Group at the Galveston Laboratory successfully mated and spawned several *Penaeus stylirostris* during August 1979. The hatching success of viable eggs, however, was poor and resulting nauplii appeared weak and did not survive past the protozoeal stage.

In an effort to increase hatching success, all water used for spawning was filtered through a 5 μ bag and treated with 5 mg/liter Erythromycin and 0.01 g/liter EDTA. A single airlift pump was installed in the center of the spawning tanks to create an upwelling and prevent settling of the eggs.

After this system was installed, those females with attached spermatophores were captured and placed in the individual spawning tanks. Hatching success increased to approximately 80%, resulting in apparently normal healthy nauplii. All nauplii used in our hatchery experiments were produced using the above described spawning system.

EXPERIMENT I

On August 17, 1979, two *P. stylirostris* were successfully mated and spawned resulting in an estimated 350,000 nauplii that were placed in one hatchery tank three days later. The hatchery water was treated and the tank set up as previously described. The objective of this study was to provide postlarvae for stocking in semi-closed intensive culture raceways. The hatchery log from Experiment I is presented in Appendix I.

After transferring the nauplii to the hatchery tank on August 20, frozen *Skeletonema costatum* was fed. Examination of the larvae the next

morning showed that they had metamorphosed to the protozoea stage and were actively feeding on the algae. Routine observations the following night showed that the diatom (*S. costatum*) was beginning to clump in the hatchery tank. Instead of individual cells or small chains of 2 to 3 cells, there were large masses of 100 cells or more. In an effort to provide sufficient food in the tank for metamorphosis to protozoea II, the level of algae was increased.

During the morning of August 23, there was very heavy clumping of the frozen alga in the hatchery tank, and the shrimp population had not metamorphosed. At that time, we continued feeding *S. costatum*, and we added the frozen alga, *Thalassiosira* sp., at a level of 5,000 cells/ml. At 1630 hours that day, both the *S. costatum* and the *Thalassiosira* sp. had formed large clumps. No explanation for this phenomenon is proposed at this time.

Shortly after 1630 hours on August 23, compressed bakers' yeast cake was prepared as previously described and introduced into the hatchery tank. Examination of hatchery water and larval shrimp that night showed that the yeast was being eaten (Appendix I) and the shrimp had metamorphosed to protozoea II. Thereafter, until the shrimp metamorphosed to protozoea III, frozen *S. costatum* and bakers' yeast were both fed.

After metamorphosis to protozoea III, *S. costatum* was replaced with *Tetraselmis chuii*, but feeding with the yeast was continued (morning of August 26 to evening of August 26). With the introduction of *T. chuii*, the dinoflagellate (*Oxyrrhis* sp.) began to multiply in the hatchery tank. A population of 2,500 *Oxyrrhis* per ml was counted in the hatchery tank at 1600 hours on August 25. *Oxyrrhis* actively feeds on the frozen *T. chuii* and is a serious competitor with the larval shrimp for this foodstuff. A volume of 200 liters of water was changed in the hatchery tank on August 25 and 26 in an effort to reduce the dinoflagellate population.

The larval shrimp had metamorphosed to the mysis stage on the morning of August 26 and frozen *Artemia* nauplii, at a level of 2 per ml, were fed. Examination of the larvae showed they were actively feeding upon the frozen *Artemia*. Prepared decapsulated *Artemia* cysts, along with frozen *Artemia* nauplii, were fed on the morning of August 27. Food counts at noon of that day (Appendix I) indicated that the shrimp had not eaten the decapsulated cysts but were still "grazing" upon the frozen *Artemia*. The increased number of *Artemia* cysts (Appendix I) in the hatchery tank may have been due to the addition of unhatched cysts with the frozen *Artemia* nauplii. At 1400 hours of that day, freshly hatched live *Artemia* nauplii were fed to the shrimp at a level of 2 per ml. The prepared decapsulated *Artemia* cysts, frozen *Artemia*, and freshly hatched live *Artemia* nauplii were fed to the larval shrimp throughout the remainder of the experiment (Appendix I).

The hatchery tanks of Experiment I were harvested on August 31, resulting in a final tank density of 142.5 larvae per liter and a survival rate of 81%. The 285,000 postlarvae averaging 6 mm total length were used to stock the semi-closed, recirculating grow-out raceways at our East Lagoon facility. The results of the grow-out phase will be reported at a later date.

EXPERIMENT II

This experiment was conducted to test a UV germicidal lamp as a method of control for *Oxyrrhis* sp. Two identical fiberglass bioassay tanks were each filled with 50 liters of water known to be contaminated with *Oxyrrhis*. Each tank was treated identically, except Tank II was fitted with the UV germicidal lamp, while Tank I received no external treatment. The results of this experiment are shown in Table 1.

Table 1. Log of Experiment II, Comparing the Effect of UV Light on Water Contaminated with *Oxyrrhis* sp.

Date, 1979		Control Tank I (no UV light)			Tank II (with UV light)		
		Tetraselmis/ml			*Tetraselmis*/ml		
Day	Hour	Standing	Feeding	*Oxyrrhis*/ml	Standing	Feeding	*Oxyrrhis*/ml
8/30	0800		5,000	8,750		5,000	8,750
	1000	2,500	10,000	15,000	2,500	10,000	15,000
						UV Light On	
	1230	8,250		40,000	10,000		No live *Oxyrrhis*
	1300		10,000		10,000		
	2200	2,500		45,000	10,000		No live *Oxyrrhis*
8/31	0800	0		55,000	10,000		No live *Oxyrrhis*
				Experiment Terminated			

Tetraselmis chuii was added to each tank at a level of 5,000 cells/ml. The population density of the dinoflagellate was determined to be 8,750/ml in each tank. It was observed that the swimming action of *Oxyrrhis* in Tank II had been reduced, while in the control they were still active and the population appeared to be increasing. In Tank I at 2200 hours the dinoflagellate population had increased to 45,000/ml and was rapidly removing the *T. chuii* from the water column. In Tank II, the *Oxyrrhis* were all dead and the algal level was constant. On the morning of August 31, the dinoflagellate population in Tank I had increased to 55,000/ml and all the frozen *T. chuii* had been consumed. In Tank II, however, there were no *Oxyrrhis* observed and the cell count of the frozen alga was still constant.

EXPERIMENTS III THROUGH X

Eight hatchery experiments were set up to determine if bakers' compressed yeast cake could be substituted for algae. The larval shrimp did not metamorphose beyond protozoea II and each experiment ended in failure. Several of the failures were attributed to equipment failure; however, a noteworthy observation on the effect of water temperature was made. The water temperature in Experiment VIII was 26.5°C, due to thermostat malfunction, while in Experiment IX the temperature was 28°C. Both tanks were stocked with larvae from the same spawn and, theoretically, should have metamorphosed from the nauplii stage to the protozoea stage at the same time. Three additional days were required before protozoea larvae were observed in Experiment VIII, whereas shrimp in Experiment IX metamorphosed on schedule.

EXPERIMENT XI

In Experiment XI, ADY was tested as a substitute for frozen algae. The hatchery log from this experiment is presented in Appendix II.

On October 16, 1979, 200,000 *P. stylirostris* nauplii I larvae were added to one hatchery tank. These larvae were all from the spawn of one female. At 1700 hours on October 17, a density of 50,000 cells/ml of ADY was added to the hatchery tank. Routine observation the next morning, October 18, showed that the shrimp larvae had metamorphosed to protozoea I and were actively feeding upon the yeast. As the shrimp "grazed" the yeast, a cell density of 50,000 cells/ml was maintained by replacement.

Examination of the larvae on the morning of October 23 indicated they were in the process of metamorphosis to mysis I and by 1600 hours of that day, mysis I shrimp were identified in the tank. At that time, frozen *Artemia* nauplii, at a density of 2/ml, were added to the hatchery tank. The mysis stage shrimp began to actively graze upon the frozen *Artemia* while the protozoea III larvae continued to feed on the yeast. The frozen *Artemia* was increased to 4 ml, while the activated dry yeast was continued at 50,000 cells/ml. On the morning of October 27, 20,000 mysis II shrimp were harvested.

Live *Artemia* nauplii, at a density of 2/ml, were added to the hatchery tank when the larval shrimp began to metamorphose from mysis II to mysis III. The frozen *Artemia* were eliminated from the feeding schedule once the population of shrimp switched from frozen to live nauplii. The first post-larval shrimp were identified on the morning of October 30, 14 days after introduction of the nauplii I larvae. The following day, 133,000 postlarvae were harvested from this hatchery tank. The density of postlarvae in the hatchery tank at the time of harvest was 66.5/liter and the overall survival was 76% (including the 20,000 mysis II larvae removed earlier).

EXPERIMENT XII

Two hatchery tanks were set up on November 1 to compare feeding yeast (ADY) with frozen algae (*S. costatum*). The temperature and salinity in each tank were adjusted to 28°C and 28 ppt. Additionally, EDTA was added to each tank at 0.01 g/liter and each tank was fitted with UV lights (AN-8) operating at a pumping rate of 2 liters/min. The spawn from one female was divided and 280,000 larvae placed in hatchery Tank I and 200,000 larvae in hatchery Tank II. The larvae were naupliar stage IV at this time.

Feeding of yeast (Tank I) and algae (Tank II) commenced on November 1 at 2200 hours, at a level of 50,000 cells/ml in each tank. Both hatchery tank populations had metamorphosed to protozoea I by the morning of November 2. The larvae being fed yeast (Tank I) were active and had well formed fecal strands, while those larvae being fed frozen algae (Tank II) appeared sluggish and had interrupted fecal strands. Examination of the hatchery water in each tank showed the yeast was evenly distributed throughout the water column while the frozen algae were clumping together.

DISCUSSION

The future of shrimp mariculture depends upon the success or failure of solving a number of intricate biological problems and economic factors. The development of hardy, fast-growing, disease-resistant penaeid shrimp, readily adaptable to most artificial culture environs by genetic manipulation, depends largely upon the solving of mating, spawning, and hatchery production problems. The nauplii of those shrimp matured, mated, and spawned in captivity must be reared to postlarvae to have any hope of closing the life cycle under controlled conditions.

Several problems were identified in our larval culture systems during the development at the Galveston Laboratory of a technique to mature, mate, and spawn *P. stylirostris*. The method initially used to hatch large masses of viable shrimp was apparently deficient and was corrected by using airlift pumps and the addition of EDTA and Erythromycin. Frozen single cell algae, used for years in the Galveston culture technique, inexplicably clumped, or were bound together in large cell masses, making the alga cells unavailable to the larval shrimp. This problem was reduced by the substitution of bakers' activated dry yeast in place of algae as foodstuff for larval shrimp. Biologically, the studies reported have demonstrated that yeast may be used as a reliable foodstuff for penaeid shrimp culture and perhaps decapod crustacean larvae in general. Economically, our data indicate that using the "Galveston technique" to rear 200,000 nauplii to postlarvae, the algae cost would be approximately $200.00. The same number of larvae could be produced with bakers' yeast at a cost of only $1.00.

All foodstuff (algae, yeast, and *Artemia* nauplii) used for larval penaeid shrimp in the Galveston hatchery system is fed dead through mysis II stage. The larval shrimp apparently feed as well on dead foodstuff as on live and, in some instances, appear to actually feed better on the dead material.

The use of frozen or dead food provides a method for control of food levels in the hatchery tank and also provides a gauge of the amount of food being eaten. Additionally, contaminants such as the dinoflagellate reported in this paper and other unwanted biological organisms are easily recognized in the hatchery tank when dead foodstuff is used. When a biological and/or chemical contaminant is identified soon enough, a solution to the problem can be developed quickly, such as the elimination of the dinoflagellate from the hatchery tanks with the use of a small UV germicidal lamp.

The techniques and methods described in this paper await further testing and replication to determine their real value. The experimental modifications, however, did increase the production of apparently normal healthy postlarvae during these studies and will be used in future hatchery studies at Galveston.

SUMMARY

Observations and data presented in this paper show that hatching success of viable eggs increased to approximately 80% by the addition of an airlift pump to spawning tanks and treatment of the water with EDTA and Erythromycin. The physical description and method of harvesting nauplii from spawning tanks are presented.

A water temperature of 28°C is ideal for culturing larvae of *P. stylirostris* to postlarvae, as it was shown that a temperature of 26.5°C retarded metamorphosis from nauplii V to protozoea I. Additionally, the UV lamp (Hawaiian Marine Imports, Inc., Model AN-8) at a germicidal intensity of 74,300 microwatts/cm^2 (2537 Angstroms), and a pumping rate of 2 liters/min, was shown to control the contaminating dinoflagellate (*Oxyrrhis* sp.) in the 2000 liter hatchery tanks.

Although some problems were encountered when feeding bakers' yeast in the compressed cake form, the bakers' yeast in the dehydrated dry yeast (ADY) form works well as a replacement for frozen algae. Using yeast, the larval population reached the post-larval stage in 10 days.

Frozen *Artemia* nauplii, live *Artemia*, and decapsulated *Artemia* cysts were accepted and readily eaten. During the course of the experiments it was observed that, at times, some of the decapsulated cysts had hatched; however, the percentage was low. Frozen *Artemia* nauplii are easier to prepare, feed, and monitor than decapsulated cysts. The shrimp populations readily accepted and "grazed" live *Artemia* nauplii during mysis III and post-larval stages during this study.

ACKNOWLEDGMENTS

During the course of this study, there were five students in resident training at the Galveston hatchery. We take this opportunity to express our gratitude and appreciation for the long hours worked and dedication shown by these people during 1979. The trainees and places of residence were: Anaxis Alvarez (Panama), Gary Mendenhall (Michigan), Tom Patterson (Florida), Tom Wallace (Florida), and Chin Ho Quek (Singapore).

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Appendix I. Daily Log of Hatchery Experiment I. Nauplii (N), Protozoea (P), Mysis (M), Postlarvae (PL), *Skeletonema costatum* (S), *Thalassiosira* sp. (Th), *Tetraselmis chuii* (T), Compressed Yeast (CY), liter (ℓ). Hatchery Tank I, Genus specie *Penaeus stylirostris*, Maturation Tank 2-3 (2 ♀'s).

Date, 1979 Day	Hour	Larval stage	Larval count	Cells per ml Residual	Cells per ml Feeding	*Artemia* per ml Frozen Residual	*Artemia* per ml Frozen Feeding	*Artemia* per ml Decapsulated Residual	*Artemia* per ml Decapsulated Feeding	*Artemia* per ml Live Residual	*Artemia* per ml Live Feeding	Remarks
8/20	0800											
	1530											32 ppt, 28°C
	1600	N IV	350,000		100,000 S							Larvae in tank
	2230	N V		80,000 S								
8/21	0800	P I		35,000 S	50,000 S							
	0815				50,000 S							
	0820			130,000 S								
	1015				50,000 S							
	1020	P I		180,000 S								
	1430		206,000									
	2145			30,000 S	100,000 S							
	2245				50,000 S							
8/22	0800	P I	140,000	40,000 S	100,000 S							
	1530			30,000 S								32 ppt, 28°C
	1545			75,000 S								
	2030		192,000	40,000 S	200,000 S							
	2215			245,000 S								
8/23	0800	P I-II		30,000 S	5,000 Th							Cells clumping
	0900				100,000 S							Heavy clumping
	1630			5,000 Th	100,000 CY							
												Skele. clumping
	2130	P II		55,000 CY								*Thala.* clumping
	2200				100,000 CY							
8/24	0800	P II		70,000 CY								
				50,000 S								*Skele.* clumping
	0845		200,000		50,000 S							*Thala.* clumping
	0900				100,000 CY							
	1400			100,000 S								
				160,000 CY								Heavy clumping
	1620	P II-III	165,000	16,250 CY	50,000 S							
				16,250 CY	100,000 CY							
	2300			80,000 S	50,000 CY							
				105,000 CY	50,000 CY							32 ppt, 28°C

Appendix I (continued)

Date, 1979 Day	Hour	Larval stage	Larval count	Cells per ml Residual	Cells per ml Feeding	*Artemia* per ml Frozen Residual	*Artemia* per ml Frozen Feeding	*Artemia* per ml Decapsulated Residual	*Artemia* per ml Decapsulated Feeding	*Artemia* per ml Live Residual	*Artemia* per ml Live Feeding	Remarks
8/25	0800	P III	200,000	65,000 CY								Larvae active
	0900				5,000 T							
	1200				5,000 T							
	1600	P III		5,000 T								2500 *Oxyrrhis* per ml
				30,000 S								
				20,000 CY	100,000 CY							
	2300			20,000 S								
				50,000 CY	100,000 CY							Change 200 ℓ H_2O
	2330			288,000 CY								
				2,500 T	5,000 T							
8/26	0800	P III M I	168,000	118,000 CY								Change 200 ℓ H_2O
				4,000 T								
				15,000 S								
	1000	M I			100,000 CY		2.0					
	1800		200,000	160,000 CY								
				60,000 S								
	1815					.05						
	1830						4.0					
	1840					4.2						
8/27	0800	M I	200,000	80,000 CY		.001	4.0					2500 *Oxyrrhis* per ml
				20,000 S								Change 400 ℓ H_2O
	1030								2.0			
	1345					1.9		2.0				
	1400										2.0	
	1600			12,000 CY		.65		.05		2.75		
	1645								4.0			
	2225					.15		2.5		1.4	2.0	
8/28	0800	M II				.05		3.1		2.8		Change 400 ℓ H_2O
	0900						2.0					
	1400	M III				2.95		1.15		2.6		Change 400 ℓ H_2O
	1630			1,250 CY		.6	2.0	2.6		3.2		1250 *Oxyrrhis* per ml
	2200					.5		3.9		3.1		
	2230						2.0					

Appendix I (continued)

Date, 1979 Day	Hour	Larval stage	Larval count	Cells per ml Residual	Cells per ml Feeding	*Artemia* per ml Frozen Residual	*Artemia* per ml Frozen Feeding	*Artemia* per ml Decapsulated Residual	*Artemia* per ml Decapsulated Feeding	*Artemia* per ml Live Residual	*Artemia* per ml Live Feeding	Remarks
8/29	0800					0		.65		2.3		32 ppt, 28°C
	0815	M III					2.0		2.0			
	1630					.03	4.0	15.0		2.5		
	2200					.9		12.0		2.2		
8/30	0800	PL				.6	4.0	5.5		.1		Change 100 ℓ H_2O
	1400					.75	4.0	9.0		.5		
	1630					2.0	2.0	5.0		.1		
	2200					1.8	4.0	5.0				
8/31	0800					4.1	4.0					
	1400	PL	285,000									Harvest tank, 81.4% survival

Appendix II. Daily Log of Hatchery Experiment XI. Nauplii (N), Protozoea (P), Mysis (M), Postlarvae (PL), Active Dry Yeast (ADY), liter (ℓ). Hatchery Tank XI, Genus specie *Penaeus stylirostris*, Maturation Tank 4 (one ♀).

Date, 1979 Day	Hour	Larval stage	Larval count	ADY cells per ml Residual	ADY cells per ml Feeding	*Artemia* per ml Frozen Residual	*Artemia* per ml Frozen Feeding	*Artemia* per ml Decapsulated Residual	*Artemia* per ml Decapsulated Feeding	*Artemia* per ml Live Residual	*Artemia* per ml Live Feeding	Remarks
10/15	1200											28 ppt, 28°C; UV at 2 ℓ/min
10/16	1500	N I	200,000									
10/17	0800	N III										
	1700	N IV										
	2245	N V			50,000							
10/18	0730	P I	240,000	35,000	50,000							Larvae eating yeast
	1700			50,000	50,000							
	2200			50,000	50,000							
10/19	0800	P I		70,000	50,000							Larvae active
	1400			72,500								2500 *Oxyrrhis* per ml
	1700			30,000	100,000							
	2245			70,000	100,000							
10/20	0800	P II	240,000	50,000	100,000							
	1030				150,000							
	1115											UV at 2 ℓ/min
	1600			40,000	100,000							
	2200			60,000	150,000							
10/21	0900	P II		50,000	100,000							
	1700			15,000	150,000							125 *Oxyrrhis* per ml
	2215			65,000	200,000							
10/22	0800	P II-III		50,000	70,000							28 ppt, 28°C
	1500			20,000	100,000							
	1615			80,000	120,000							
	2300			30,000	150,000							
10/23	0800	P III	100,000	20,000	100,000							
	1600	P III-M I		30,000	100,000		2.0					
	2110			80,000		.4	4.0					
10/24	0800			40,000	50,000	2.0						No *Oxyrrhis*
	1300			20,000		0	2.0					
	1600			40,000	100,000	.1	4.0					
	2015			90,000		.1	4.0					

Appendix II (continued)

Date, 1979 Day	Hour	Larval stage	Larval count	ADY cells per ml Residual	ADY cells per ml Feeding	*Artemia* per ml Frozen Residual	*Artemia* per ml Frozen Feeding	*Artemia* per ml Decapsulated Residual	*Artemia* per ml Decapsulated Feeding	*Artemia* per ml Live Residual	*Artemia* per ml Live Feeding	Remarks
10/25	0800	M I	100,000	80,000		0	4.0					
	1630					0	4.0					
	2200					0	4.0					
10/26	0800					0	4.0					
	1600	M II		40,000		.2	4.0					Change 400 ℓ H_2O
	2200					.15	4.0					
10/27	0800					0	4.0					Change 800 ℓ H_2O
	0900					0	4.0					20,000 M II removed from tank
	1900					0	4.0					
10/28	0900	M III				0.1	3.0				2.0	Change 800 ℓ H_2O
	2000					0	2.0			2.0		
10/29	0800			10,000		.3	.5			1.4	3.0	
	1600					.1	3.0			.6	6.0	
	2200					1.5				2.5	2.5	
10/30	0730	M III-PL				.6				1.6	3.0	
	1600									.7	4.0	
	2200									.9	4.0	
10/31	0700	PL	133,000							.8	4.0	Harvest tank 133,000 +20,000 = 153,000
												76.5% survival